and a putative model for the transition state is proposed. This information can now be used to rationalize the energetic and conformational effects of oncogenic mutations and the binding of antibodies.

1504-Pos
Oligomerization of Membrane Receptors: FRET Analysis Using Coiled-Coil Tag-Probe Labeling and Spectral Imaging
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Oligomerization of membrane receptors in living cell membranes plays an important role in regulation of receptor activity and trafficking. Förster resonance energy transfer (FRET) techniques are often employed to detect receptor oligomerization using receptors fused with fluorescent/luminescent proteins. However, the large size of fluorescent proteins often interferes localization and function of target receptors. Furthermore, controlling the labeling ratio (donor/acceptor), which is important for analysis of FRET, is usually difficult when two fusion receptors with different colors are co-expressed. Posttranslational labeling methods using a short tag peptide and a fluorescent probe that specifically binds to the tag enable a smaller size of label and easy control of labeling ratio in multicolor labeling [1,2]. We recently developed a quick (< 1 min) and surface-specific tag-probe method using a high-affinity heterodimeric coiled-coil formation between the E3 tag (EIAALEK)3 attached to the target receptor and the Kn probes (KIAALKE)n (n = 3 or 4) labeled with a fluorophore [3]. Here we examine oligomerization of the metabotropic glutamate receptor (mGluR) using this novel technique. The receptors were labeled with Rhodamine Green (donor) or Tetramethylrhodamine (acceptor) fluorophores. A constant FRET signal was observed for mGluRs transiently expressed in CHO cells, indicating constitutive oligomer formation. Spectral imaging and demixing of emission spectra abolish crosstalk between channels that is inevitable in conventional filter detection therefore simplify quantification of FRET efficiency from sensitized acceptor emission, allowing analysis of stoichiometry of the oligomerization.
[1] Murel et al. Nat. Mathods (2008) 5, 561-567.
[2] Meyer et al. PNAS (2006) 103, 2138-2143.
[3] Yano et al. ACS Chem. Biol. (2008) 3, 341-345

1505-Pos
How does the State of Aggregation of Rhodopsin in Retinal Discs Influence the Variability of its Activated Life Time?
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Single photon responses (SPRs) in the retinal rod are less variable than expected assuming that deactivation of the receptor rhodopsin (R*) occurs in a single memoryless step. It has been suggested that SPRs reproducibility can be explained by a sequential increase of affinity of R* to the protein involved in its deactivation arrestin (Arr) which leads to a reduction in the life-time variability of R*. This increase in affinity is promoted by a serial phosphorylation of rhodopsin catalyzed by rhodopsin- kinase (RK).
This deactivation mechanism has been tested successfully by means of stochastic simulation assuming rapid diffusion of all signaling molecules. However, evidence suggests that, in native rod discs, rhodopsin is found forming dimers organized in paracrystalline arrays covering about half of the membrane surface.
In this work, we test the hypothesis that packing induced crowding effects, in conjunction with the competitive interactions between R* an the other proteins involved in the signaling cascade (G protein (G), RK, and Arr) will influence the variability of the half-time of R*. In particular, we explore whether the local decrease of inactivated G around R* (as it becomes activated by R*) facilitates interactions of the receptor with Arr and RK, increasing the probability of R* deactivation. This would then lead to a reduction in its trial to trial variation.
In order to explore these issues, we implement a mesoscopic Monte Carlo simulation in a two-dimensional grid representing the membrane, and follow the stochastic encounters and reactions between the species involved in the signal cascade. We perform the simulations and present data on the variability of the half life of R* under two scenarios: rapid diffusion of all proteins, and immobile paracrystalline arrays of rhodopsin.

1506-Pos
Comparative Interaction of Tricyclic Antidepressants and Mecamlyamine with the Human α4β2 Nicotinic Acetylcholine Receptor
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We compared the interaction of tricyclic antidepressants (TCAs) with that for the noncompetitive antagonist mecamylamine with the human (h) α4β2 nicotinic acetylcholine receptor (AChR) in different conformational states, by using functional and structural methods. The results established that: **(a)** TCAsinhibit (±)-epibatidine-induced Ca^{2+} influx in HEK293-hα4β2 cells with potencies that are in the same concentration range (IC_{50} = 2.2-6.8 μM) as that for mecamylamine (IC_{50} = 3.0 ± 0.7 μM), **(b)** [^{3}H]imipramine binds to a single binding site located in the hα4β2 AChR ion channel with relatively high affinity (K_d = 0.83 ± 0.08 μM), **(c)** TCAsinhibit [^{3}H]imipramine binding to hα4β2 AChRs with affinities (K_i = 1.0-2.1 μM) higher than that for mecamylamine (K_i = 143 ± 31 μM), **(d)** imipramine and mecamylamine do not differentiate between desensitized and resting AChRs, **(e)** imipramine interacts with the desensitized AChR mainly by an entropy-driven process, whereas the interactions with the resting AChR are mediated by a combination of enthalpic and entropic components, and **(f)** neutral imipramine and mecamylamine interact with a domain formed between the leucine (position 9') and valine (position 13') rings by van der Waals contacts, whereas protonated mecamylamine interacts electrostatically with the outer ring (position 20'). Our data indicate that although TCAs interact with a binding domain located between the leucine and valine rings, and mecamylamine predominantly acts at the outer ring and by intercalating between two M2 segments, both drugs may efficiently inhibit the ion channel.

1507-Pos
Common Dynamic Behavior of Inactive G-Protein Coupled Receptor Structures for Diffusible Ligands
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The crystal structures of three different family A G-protein coupled receptors (GPCRs) for diffusible ligands, i.e., engineered forms of human β2-adrenergic (B2AR) and adenosine A2A receptors (A2AR), as well as a turkey β1-adrenergic receptor (B1AR) mutant, have recently become available in the literature. Although the overall helical-bundle topology is conserved among these three inactive GPCR structures, several differences emerge from their comparison, particularly at TM1, the extracellular region, the cytoplasmic side of helices TM5-TM7, the ligand-binding pocket, and the long loop regions. Although one cannot exclude the possibility that crystallographic artifacts may be causing some of these structural differences, it remains to be addressed whether these different GPCR structures would share a common dynamic behavior during molecular dynamics simulations in an explicit lipid-cholesterol-water environment. The results of nanosecond-scale simulations of ligand-free inactive crystallographic forms of B2AR, B1AR, and A2AR were analyzed in terms of inter-residue contact variability over time. Contacts that remained in place during most of the simulations were recognized as stable contacts. Among them, the most stable contacts were found to be common among the three GPCR structures, and to involve residues that are conserved among family A GPCRs. We propose that these stable contacts define a common dynamic behavior of inactive GPCR structures for diffusible ligands, and are therefore important for keeping the receptors in an inactive state.

1508-Pos
Protonation Switches in GPCR Activation: Physiologically Significant Rhodopsin Photointermediates
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Rhodopsin is a paradigm for GPCRs, yet unlike other class A members, its bound chromophore's UV/vis absorbance provides excellent time-resolved information about GPCR activation steps. At least three species equilibrate on the millisecond time scale after rhodopsin photoexcitation in a membrane lipid environment. The first equilibrium is pH-independent and involves Meta I_{480}, the visible absorbing, protonated Schiff base (PSB) species, and Meta IIa, the UV absorbing, deprotonated SB species. The second equilibrium, involving spectrally silent proton uptake by Meta IIa to produce Meta IIb, accounts for the fact that low pH causes anomalous disappearance of the protonated SB species, Meta I_{480}. The equilibria affect production of the G protein-activating species R* and are of great interest. However they must be studied promptly because inactivation steps follow, and long illumination increases secondary photolysis of photoproducts. We used time-resolved absorbance measurements of bovine rhodopsin on the microsecond-to-hundred millisecond